

WHAT IS CLAIMED IS:

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A resin-protein/peptide complex which comprises a resin and a target protein of peptide bound thereto wherein said resin comprises

- (a) a solid support matrix; and
- b) selected ionizable ligand covalently attached to the matrix wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength.
- 2. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin.
- 3. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is negatively charged at the pH where the target protein or peptide is desorbed from the resin.
- 4. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises an ionizable functional group directly attached to the solid support matrix.
- 5. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises a spacer arm and at least one ionizable functionality wherein the ionizable functionality is attached to the solid support matrix via the spacer arm.



The resin-protein/septide complex of Claim 1 wherein the solid support matrix is functionalized with carboxyl groups which are protonated at the pH where the target protein or peptide is bound to the resin and are deprotonated and negatively charged at the pH where the target protein or peptide is desorbed from the resin.

7. The resin-protein/peptide complex of Claim 1 wherein the resin further comprises non-ionizable ligands.

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- 8. The resin-protein/peptide complex of Claim 7 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater than 0% to about 80%.
- 9. The resin-protein/peptide complex of Claim 8 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater than 0% to about 40%.
- 1 10. The resin-protein/peptide complex of Claim 1 wherein the solid 2 support matrix is cross-linked.
- 1 11. The resin-protein/peptide complex of Claim 1 wherein the resin contains from about 0.05 mmol to about 0.5 mmol ionizable ligand per ml of the solid support matrix prior to covalent attachment of any non-ionizable ligand.
- 1 12. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix is non-ionizable.



- 13. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix contains ionizable functionality which functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin.
- 14. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the same polarity as the net electrostatic charge on the target protein or peptide at the pH of desorption.
- 15. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption.

16. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

- a) a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof wherein the ionizable functionality is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin; and
 - b) optionally a non-ionizable ligand covalently attached thereto.

wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength.

17. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality is electrostatically uncharged at the pH where the target

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3 protein or peptide is bound to the resin and is positively charged at the pH 4 where the target protein or peptide is desorbed from the resin. 1 18. The resin-protein/peptide complex of Claim 16 wherein the 2 ionizable functionality is electrostatically uncharged at the pH where the target 3 protein or peptide is bound to the resin and is negatively charged at the pH 4 where the target protein or peptide is desorbed from the resin. 1 19. The resin-protein/peptide complex of Claim 16 wherein the 2 ionizable functionality comprises amino groups covalently attached in the 3 backbone of the solid support matrix. 1 20. The resin-protein/peptide complex of Claim 16 wherein the solid 2 support matrix is cross-linked. 1 21. The resin-protein/peptide complex of Claim 16 wherein the resin 2 contains from about 0.05 mmol to about 0.5 mmol non-ionizable ligand per ml 3 of the solid support matrix. 1 22. The resin-protein/peptide complex of Claim 16 wherein the 2 electrostatic charge induced on the resin of the resin-protein/peptide complex is 3 of the same polarity as the net electrostatic charge on the target protein or 4 peptide at the pH of desorption. 1 23. The resin-protein/peptide complex of Claim 16 wherein the

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peptide at the pH of desorption.

electrostatic charge induced on the resin of the resin-protein/peptide complex is

of the opposite polarity from the net electrostatic charge on the target protein or

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- 24. A method for binding and recovering a target protein or peptide from an aqueous medium comprising the target protein or peptide which method comprises:
- a) contacting the medium with a resin under conditions sufficient to allow the target protein or peptide to bind to the resin wherein said resin comprises a solid support matrix and selected ionizable ligand covalently attached to the matrix wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength;
- b) separating the resin containing the bound target protein or peptide from the other components of the medium to produce a resin-protein/peptide complex; and
- c) desorbing the bound target protein or peptide from the complex by contacting the complex with a desorbing solution having a pH which induces an electrostatic charge on the resin wherein the induced charge is of the same polarity as the net charge on the target protein or peptide at the pH of the desorbing solution.
- 25. A method for binding and recovering a target protein or peptide from an aqueous medium comprising the target protein or peptide which method comprises:
- a) contacting the medium with a resin under conditions sufficient to allow the target protein or peptide to bind to the resin wherein said resin comprises a solid support matrix and selected ionizable ligand covalently attached to the matrix wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin and further wherein about 50

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l 1	percent or more of the target protein or peptide in an aqueous medium binds to
12	the reson when the aqueous medium has either a high or a low ionic strength;
13	b) separating the resin containing the bound target protein or peptide
14	from the other components of the medium to produce a resin-protein/peptide
15	complex; and
б	c) desorbing the bound target protein or peptide from the complex by
17	contacting the complex with a desorbing solution having a pH which induces an
8	electrostatic charge on the resin wherein the induced charge is of the opposite
9	polarity from the net charge on the target protein or peptide at the pH of the
20	desorbing solution.
1	26. The method of either Claim 24 or Claim 25 wherein the induced
2	charge on the resin is a positive charge.
1	27. The method of either Claim 24 or Claim 25 wherein the induced
2	charge on the resin is a negative charge.
1	28. The method of either Claim 24 or Claim 25 wherein the aqueous
2	medium is contacted with the resin in a stirred batch process.
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1	29. The method of either Claim 24 or Claim 25 wherein the aqueous
2	medium is contacted with the resin in a chromatography column.
1	30. The method of claim 29 wherein the aqueous medium is contacted
2	with the resin in a fluidized expanded bed.
1	31. The method of Claim 29 wherein the column is a radial flow
2	column.
l	32. The method of either claim 24 or Claim 25 wherein the aqueous

medium is a crude fermentation broth.

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	l	33. The method of Claim 32 wherein the crude fermentation broth
	2	comprises a protein selected from the group consisting of chymosin and
	3	subtilisin.
	1	34. The method of either Claim 24 or Claim 25 wherein binding of the
	2	target protein or peptide to the resin is conducted at a pH of from 2 to 12.
	l	35. The method of Claim 34 wherein binding of the target protein or
	2	peptide to the resin is conducted at a pH of from 5 to 9.
	1	36. The method of Claim 34 wherein desorption of the target protein or
	2	peptide from the resin is conducted at a pH within the range of from 2 to 12
	3	but at a pH different from that employed to bind the target protein or peptide
	4	onto the resin.
	1	37. The method of Claim 35 wherein desorption of the target protein or
	2	peptide from the resin is conducted at a pH within the range of from 5 to 9 but
	3	at a pH different from that employed to bind the target protein or peptide onto
	4	the resin.
	1	38. The method of Chaim 34 wherein the pH of the aqueous mixture is
	2	adjusted to from about pH 2 to about pH 12 before contacting the mixture with
	3	the resin.
	I	39. A method for binding and recovering a target protein or peptide.
	2	from an aqueous medium comprising the target protein or peptide which
	3	method comprises:
	4	a) contacting the medium with a resin under conditions sufficient to
	5	allow the target protein or pentide to hind to the resin wherein said resin

comprises a solid support matrix having a selected ionizable functionality

incorporated into the backbone thereof wherein the ionizable functionality is

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- selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength;
- b) separating the resin containing the bound target protein or peptide from the other components of the medium to produce a resin-protein/peptide complex; and
- c) desorbing the bound target protein or peptide from the complex by contacting the complex with a desorbing solution having a pH which induces an electrostatic charge on the resin wherein the induced charge is of the same polarity as the net charge on the target protein or peptide at the pH of the desorbing solution.
- 40. A method for binding and recovering a target protein or peptide from an aqueous medium comprising the target protein or peptide which method comprises:
- a) contacting the medium with a resin under conditions sufficient to allow the target protein or peptide to bind to the resin wherein said resin comprises a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof wherein the ionizable functionality is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein greater than 50 percent of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or a low ionic strength;
- b) separating the resin containing the bound target protein or peptide from the other components of the medium to produce a resin-protein/peptide complex; and

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- c) desorbing the bound target protein or peptide from the complex by contacting the complex with a desorbing solution having a pH which induces an electrostatic charge on the resin wherein the induced charge is of the opposite polarity from the net charge on the target protein or peptide at the pH of the desorbing solution.
- 1 41. The method of either Claim 39 or Claim 40 wherein the induced 2 charge on the resin is a positive charge.
- 1 42. The method of either Claim 39 or Claim 40 wherein the induced 2 charge on the resin is a negative charge.
 - 43. The method of either Claim 39 or Claim 40 wherein the aqueous medium is contacted with the resin in a stirred batch process.
 - 44. The method of either Claim 39 or Claim 40 wherein the aqueous medium is contacted with the resin in a chromatography column.
 - 45. The method of Claim 44 wherein the aqueous medium is contacted with the resin in a fluidized bed.
 - 46. The method of Claim 44 wherein the column is a radial flow column.
 - 47. The method of either Claim 39 or Claim 40 wherein the aqueous medium is a crude fermentation broth.
- 1 48. The method of Claim 47 wherein the crude fermentation broth comprises a target protein selected from the group consisting of chymosin and subtilisin.

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1	49. The method of either Claim 39 or Claim 40 wherein binding of the
2	target protein or peptide to the resin is conducted at a pH of from 2 to 12.
1	50. The method of Claim 49 wherein binding of the target protein or
2	peptide to the resin is conducted at a pH of from 5 to 9.
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1	51. The method of Claim 49 wherein desorption of the target protein or
2 -	peptide from the resin is conducted at a pH within the range of from 2 to 12
3	but at a pH different from that employed to bind the target protein or peptide
4	onto the resin.
1	52. The method of Claim 0 wherein desorption of the target protein or
2	peptide from the resin is conducted at a pH within the range of from 5 to 9 but
3	at a pH different from that employed to bind the target protein or peptide onto
4	the resin.
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1	53. The method of Clain 49 wherein the pH of the aqueous mixture is
2	adjusted to from about pH 2 to about pH 12 before contacting the mixture with
3	the resin.
1	54. A method for separating a target protein or peptide from an
2	aqueous medium comprising the target protein or peptide which method
3	comprises contacting the medium with a esin under conditions sufficient to
4	allow the target protein or peptide to bind to the resin so as to form a resin-
5	protein/peptide complex as described in either Claim 1 or Claim 16.
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